

KAMIYA BIOMEDICAL COMPANY

Human MMP-2 ELISA

**For the quantitative determination of MMP-2
in human cell culture supernates, serum and plasma (heparin)**

Cat. No. KT-1391

For Research Use Only. Not for diagnostic use in the U.S.

PRODUCT INFORMATION**Human MMP-2 ELISA
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INTRODUCTION

Type IV collagenase, 72-kD, is officially designated matrix metalloproteinase-2 (MMP2). It is also known as gelatinase, 72-kD. MMP-2 plays an essential role in angiogenesis and arteriogenesis, two processes critical to restoration of tissue perfusion after ischemia. MMP-2 expression is increased in tissue ischemia, but the responsible mechanisms remain unknown. Matrix metalloproteinases (MMPs) catalyze extracellular matrix degradation. Control of their activity is a promising target for therapy of diseases characterized by abnormal connective tissue turnover. MMPs are expressed as latent proenzymes that are activated by proteolytic cleavage that triggers a conformational change in the propeptide (cysteine switch). The structure of proMMP-2 reveals how the propeptide shields the catalytic cleft and that the cysteine switch may operate through cleavage of loops essential for propeptide stability. The gene is localized to 16q21 using somatic cell hybrids and in situ hybridization. The calibrator product used in this kit is recombinant human MMP-2, consisting of 631 amino acids with the molecular mass of 71 kDa. The detected MMP-2 includes zymogen and active enzyme.

PRINCIPLE

The human MMP-2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for MMP-2 has been precoated onto 96-well plates. Calibrators (NSO, A30-C660) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for MMP-2 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human MMP-2 amount of sample captured in plate.

COMPONENTS

1. 96-well plate precoated with anti-human MMP-2 antibody: 1
2. lyophilized recombinant human MMP-2 calibrator: 10 ng/tube x 2
3. biotinylated anti-human MMP-2 antibody: 130 μ L (dilution 1:100)
4. Avidin-Biotin-Peroxidase Complex (ABC): 130 μ L (dilution 1:100)
5. sample diluent buffer: 30 mL
6. antibody diluent buffer: 12 mL
7. ABC diluent buffer: 12 mL
8. TMB color developing agent: 10 mL
9. TMB stop solution: 10 mL

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader in standard size.
2. Automated plate washer.

3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

-Preparation of 0.01 M **TBS**: Add 1.2 g Tris, 8.5 g NaCl; 450 μ L of purified acetic acid or 700 μ L of concentrated hydrochloric acid to 1,000 mL H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

-Preparation of 0.01 M **PBS**: Add 8.5 g sodium chloride, 1.4 g Na₂HPO₄ and 0.2 g NaH₂PO₄ to 1,000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

PRECAUTIONS

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using calibrators and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both calibrator and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

REAGENT PREPARATION

1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 4°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

-**Cell culture supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

-**Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.

-**Plasma**: Collect plasma using **heparin** as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the calibration curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

-**High target protein concentration (100-1,000 ng/mL)**. The working dilution is 1:100. i.e. Add 1 μ L sample into 99 μ L sample diluent buffer.

-**Medium target protein concentration (10-100 ng/mL)**. The working dilution is 1:10. i.e. Add 10 μ L sample into 90 μ L sample diluent buffer.

-**Low target protein concentration (156-10,000 pg/mL)**. The working dilution is 1:2. i.e. Add 50 μ L sample to 50 μ L sample diluent buffer.

-**Very Low target protein concentration (\leq 156 pg/mL)**. No dilution necessary, or the working dilution is 1:2.

3. Reagent Preparation and Storage

A. Reconstitution of the human MMP-2 calibrator: MMP-2 calibrator solution should be prepared no more than 2 hours prior to the experiment. Two tubes of MMP-2 calibrator (10 ng per tube) are included in each kit. Use one tube for each experiment.

- a. 10,000 pg/mL of human MMP-2 calibrator solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- b. 5,000 pg/mL→156 pg/mL of human MMP-2 calibrator solutions: Label 6 Eppendorf tubes with 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 10,000 pg/mL MMP-2 calibrator solution into 1st tube and mix. Transfer 0.3 mL from 1st tube to 2nd tube and mix. Transfer 0.3 mL from 2nd tube to 3rd tube and mix, and so on.

Note: The calibrator solutions are best used within 2 hours. The 10 ng/mL calibrator solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human MMP-2 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- a. The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- b. Biotinylated anti-human MMP-2 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-human MMP-2 antibody to 99 µL antibody diluent buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- a. The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC diluent buffer.)

STORAGE

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

ASSAY PROTOCOL

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Calibrator MMP-2 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of MMP-2 amount in samples.

1. Aliquot 0.1 mL per well of the 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL human MMP-2 calibrator solutions into the precoated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of human cell culture supernates, serum or plasma (heparin) to each empty well. **See “Sample Dilution Guideline” above for details.** It is recommended that each human MMP-2 calibrator solution and each sample be measured in duplicate.

2. Seal the plate with the cover and incubate at 37°C for 90 min.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. Add 0.1 mL of biotinylated anti-human MMP-2 antibody working solution into each well and incubate the plate at 37°C for 60 min.

5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 mL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)

6. Add 0.1 mL of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).

8. Add 90 µL of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human MMP-2 calibrator solutions; the other wells show no obvious color).

9. Add 0.1 mL of prepared TMB stop solution into each well. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative O.D.₄₅₀) = (the O.D.₄₅₀ of each well) – (the O.D.₄₅₀ of Zero well). The calibration curve can be plotted as the relative O.D.₄₅₀ of each calibrator solution (Y) vs. the respective concentration of the calibrator solution (X). The human MMP-2 concentration of the samples can be interpolated from the calibration curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

SUMMARY

1. Add samples and calibrators and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 min.
5. Add TMB stop solution and read.

PERFORMANCE CHARACTERISTICS

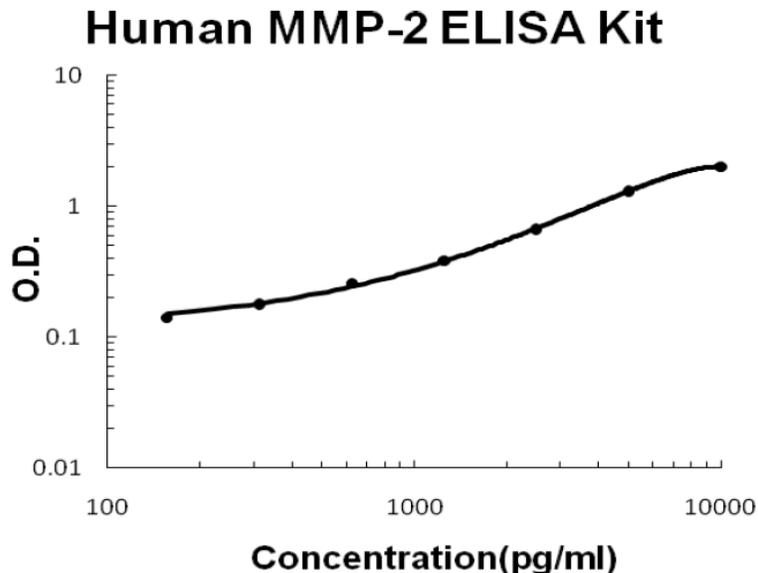
Typical Data Obtained from Human MMP-2

(TMB reaction incubate at 37°C for 25 min)

Concentration(pg/ml)	0	156	312	625	1250	2500	5000	10,000
O.D	0.100	0.141	0.178	0.259	0.383	0.666	1.305	2.001

Typical Human MMP-2 ELISA Kit Calibration Curve

This calibration curve was generated for demonstration purpose only. A calibration curve must be run with each assay.



Range: 156 pg/mL - 10,000 pg/mL

Sensitivity: <10 pg/mL

Specificity: Natural and Recombinant Human MMP-2

Cross-reactivity: No detectable cross-reactivity with other relevant proteins.

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	1526	3864	6733	1705	4012	6928
Standard deviation	74.8	216.4	444.4	97.2	256.8	512.7
CV(%)	4.9	5.6	6.6	5.7	6.4	7.4

FOR RESEARCH USE ONLY**KAMIYA BIOMEDICAL COMPANY**

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